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14. ABSTRACT
This project was designed to evaluate a novel approach in the treatment of heat-related injury, in an animal model, and hypothesized that the identification of changes in ANS regulation associated with chronic exertional heat stress may hold a key to: 1) determining a primary cause of heat-related maladies, and 2) designing effective prevention and/or treatment approaches.

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1. INTRODUCTION

Conditions that Soldiers regularly encounter - repeated exposure to environmental extremes (e.g., high ambient temperatures), along with circumstances that significantly raise body temperature (e.g., marching/running, protective clothing, placement in confined spaces) - can have serious health consequences for Warfighters, negatively impact their performance, and produce a heavy toll in terms of lost man-hours. Fundamental research efforts aiming to enhance the level of a Soldier's adaptability (i.e., stress resistance or resiliency) to environmental extremes have been defined as a research area of the highest importance by the Department of Defense. Soldiers continue to be placed in situations that quickly cause exertional heat injury or heat stroke. Repeated exposure to such extreme conditions induces profound pathophysiological changes that can subsequently translate to serious functional and behavioral deficits. One of the key regulators of homeostasis in humans under such challenging conditions is the autonomic nervous system (ANS). The ANS serves as a primary controller of the cardiovascular (CV), respiratory, and thermoregulatory systems. Therefore, conditions that produce ANS dysfunction can profoundly impair the ability of a person to cope with both physical and mental challenges. For instance, there is striking evidence that heat stress and chronic exposure to intense physical activity cause profound autonomic imbalance, as evidenced by elevated sympathetic and reduced parasympathetic tone. Thus, it is reasonable to hypothesize that chronic (repeated) exposure to the combination of heat stress and physical activity – conditions that Soldiers face on a regular basis - can cause severe autonomic dysfunction. ANS dysfunction can directly impact CV function and the ability to regulate heart rate (HR) and blood pressure (BP). These alterations can impair physical and mental performance and increase morbidity/mortality in field settings. **Therefore, this project was designed to evaluate a novel approach in the treatment of heat-related injury, in an animal model, and hypothesized that the identification of changes in ANS regulation associated with chronic exertional heat stress may hold a key to: 1) determining a primary cause of heat-related maladies, and 2) designing effective prevention and/or treatment approaches.**

2. KEY WORDS

Heat stress; heat stroke; heat injury; exercise; exertion; heat acclimation; clonidine; lisinopril; heart rate variability; sympathetic nervous system.

3. OVERALL PROJECT SUMMARY

Listed below is an overall summary of the results obtained, as well as the key methodologies used, for the two primary tasks associated with this project. Each task is presented separately.

- **TASK 1: Characterize the extent to which repeated (i.e., chronic) exposure to conditions promoting exertional heat stress (ExHS) causes autonomic nervous system (ANS) dysfunction and organ-specific tissue damage.**

The experiments performed for these studies were chronic in nature and very demanding for research team. The protocol consists of a surgical procedure in rats (8-wk-old male Sprague-Dawley) that involved implantation of a telemetry probe for the measurement of core temperature (T_c), blood pressure (BP) and heart rate (HR), and an extended recovery period (~2 weeks). Recovery was followed by a familiarization phase, and then experimental testing (an exertional heating protocol that utilized a programmed running-wheel regimen, repeated over several days). Tests to assess changes in locomotion, motor function and cognitive function were also performed in these animals. These tests were conducted multiple times before, during and after the exertional heating period in order to obtain a temporal response pattern of potential changes in functional responses to the chronic stress challenge. The entire duration for the testing for one rat lasted approximately 28 days (surgery, recovery, familiarization, repeated challenge, post-challenge measurements and behavioral testing, tissue acquisition).

- **Outline of Experimental Protocol Employed**

The following protocols were employed:

- **Exertional Heat Stress (ExHS):** rats were run on a motorized running wheel (7-11 m/min) at 39˚C ambient temperature (T_a) until T_c reached 41.8˚C.
- **Heat Stess (HS):** rats were placed in a stationary running wheel at a T_a of 45˚C until T_c reached 41.8˚C. The higher T_a serves to achieve the target T_c within the same time period as in ExHS.
- **SHAM (control)** protocol: rats were placed in the running wheel (0 m/min) at a T_a of 23˚C for 90 min (average ExHS protocol duration).
- Study protocol: 14 days recovery from surgery, followed by continuous recordings of T_c, BP, and HR during:
  - 3 days of familiarization to running wheel and behavioral testing protocols
  - 3 days of baseline recording
  - 5 days of the ExHS, HS or SHAM protocol, conducted twice a day, 8 h apart
  - 3 days of recovery recording
- Termination of study: euthanization and tissue/blood collection
- Subjects per group: a total of 10-12 rats was used in each of the three groups.
- Data Analysis: Physiological parameters (T_c, BP, HR) and circadian rhythms were assessed for potential changes related to each intervention. These physiological measures

were then assessed with sophisticated analysis techniques (e.g., power spectral analysis) to evaluate potential autonomic nervous system alterations associated with the stress protocol. In addition, these parameters were measured for three days preceding the stress protocol so that pre- and post-challenge responses could be evaluated. This design provided additional insight into the impact of ExHS (compared to heat stress alone) on physiological (and behavioral) responses in these animals.

For each of the 11 protocol days (baseline, chronic stress, recovery), clean 1-h BP segments were obtained during the active nighttime period (to avoid confounding effects of protocols performed during daytime hours). From these 1-h BP recordings the following parameters were determined:

1. Mean values for systolic, diastolic, and mean BP, HR, and T_c.
2. Low frequency (LF) systolic blood pressure variability as an index of sympathetic modulation of vascular tone.
3. LF and high frequency (HF) heart rate variability as indices for sympathetic and parasympathetic modulation of cardiac function, respectively.
4. Time domain HR variability parameters SDNN and RMSSD as markers for overall (sympathetic and parasympathetic) and parasympathetic mediated HR variability, respectively (note: low values indicate high cardiovascular risk).

- Statistics: Statistical analysis of data was performed by two-way Analysis of Variance (ANOVA) for one repeated (time) and one independent (groups) measure.

- **Results for Task 1**
  - A steady decline in nocturnal T_c was observed over the five-day exertional (ExHS) or non-exertional heat stress (HS) protocols in rats, suggesting that heat acclimation occurred during the protocol.
  - A lack of hyperthermia was observed during the night of the first recovery day in rats exposed to ExHS and persistent hyperthermia was observed in rats exposed to HS, indicating greater heat acclimation in ExHS than in HS.
  - Reduced SDNN (overall heart rate variability) and elevated LF_{HR} (cardiac sympathetic modulation) levels were observed on the first night of recovery in ExHS but not in HS, indicating more severe cardiovascular and autonomic dysfunction in ExHS than in HS.
  - A greater LF_{SysBP} (sympathetic modulation of vascular tone) was observed in ExHS than in HS, which may contribute to the nocturnal hypertension observed in rats exposed to chronic ExHS.
  - Behavioral testing suggested that there was an increase in fatigue and/or stress levels in the ExHS and HS groups (compared to controls), with the more pronounced effects occurring in the ExHS animals (despite evidence of heat acclimation in this group).
  - There was no evidence of tissue injury (liver, kidney, heart, skeletal muscle) in selected organs upon histological evaluation.

- **Conclusions from Task 1**

  These results suggest that chronic exposure to ExHS can lead to a sustained increase in cardiac and vascular sympathetic activity despite the occurrence of exercise training effects and heat acclimation. This increase in cardiac and vascular sympathetic activity in the ExHS group potentially contributes to autonomic and cardiovascular dysfunction as suggested by reductions in heart rate variability indices (e.g., SDNN).

B. TASK 2: Determine the potential efficacy of selected drug treatments to mitigate the cardiovascular autonomic dysfunction and organ damage associated with chronic exposure to EHS-promoting conditions.

As a follow-up to Task 1 studies, where we demonstrated that repeated exposure to heat stress alone (HS) or exertional heat stress (ExHS) produce a pronounced increase in nocturnal heart rate (HR), blood pressure (BP), core temperature (T_c), and sympathetic modulation of cardiac and vascular function, the primary focus of Aim 2 was to assess the effect of the centrally acting sympatholytic drug **clonidine** and the angiotensin converting enzyme inhibitor **lisinopril** on hemodynamic, thermoregulatory, autonomic, and behavioral responses to HS and ExHS.

Clonidine is known to have central effects on thermoregulation and lisinopril has been shown to provide end-organ protection in various cardiovascular diseases. At higher doses, these medications have been prescribed to treat high blood pressure in humans and both may have relevance as potential preventative or therapeutic treatments for situations encountered by Warfighters.

- **Hypothesis**: The hypothesis being tested in Task 2 studies was that clonidine and lisinopril treatments mitigate autonomic nervous system dysfunction, improve thermoregulation, and reduce fatigue and/or anxiety in response to ExHS or HS. We also postulated that clonidine and lisinopril may modulate autonomic and thermoregulatory responses via different mechanisms.

- **Design**

The experimental design for these studies was similar to the one employed for Task 1 experiments (i.e., instrumented rats undergoing a chronic ExHS, HS, or sham protocol). The design is listed below in detail.

An experiment in a single animal lasts ~31 days from beginning to end, and included surgical implantation of a telemetry probe, recovery, a familiarization period, 6-days of baseline recordings without (3 days) and with (3 days) drug application, a 5-day repeated challenge period, and a 3-day recovery period. Clonidine (33 μg/kg/day, which is a concentration that minimally affects arterial blood pressure), lisinopril (33 μg/kg/day, also a concentration that minimally affects arterial blood pressure) or placebo were continuously administered via surgically implanted osmotic mini-pumps starting 3 days before the first ExHS, HS, or sham protocol day. On the protocol days, rats were exposed to ExHS, HS, or sham conditions twice a day (separated by 8 h). Experimental conditions were terminated once T_c reached 41.8^{o}C (ExHS and HS) or after 90 min (sham; average ExHS duration).

- **Outline of General Experimental Protocol Employed**

  The following protocols were employed:

  - **Exertional Heat Stress (ExHS):** rats were run on a motorized running wheel (7-11 m/min) at 39˚C ambient temperature (T_a) until T_c reached 41.8˚C.
  - **Heat Stess (HS):** rats were placed in a stationary running wheel at a T_a of 45˚C until T_c reached 41.8˚C. The higher T_a serves to achieve the target T_c within the same time period as in ExHS.
  - **SHAM (control)** protocol: rats were placed in the running wheel (0 m/min) at a T_a of 23˚C for 90 min (average ExHS protocol duration).
  - Study protocol: 14 days recovery from surgery, followed by continuous recordings of T_c, BP, and HR during:
    - 3 days of familiarization to running wheel and behavioral testing protocols
    - 3 days of baseline recording
    - 5 days of the ExHS, HS or SHAM protocol, conducted twice a day, 8 h apart
    - 3 days of recovery recording
  - Termination of study: euthanization and tissue/blood collection
  - Subjects per group: a total of 9-10 rats was used in each of the three groups.
  - Data Analysis: Physiological parameters (T_c, BP, HR) and circadian rhythms were assessed for potential changes related to each intervention. These physiological measures were then assessed with sophisticated analysis techniques (e.g., power spectral analysis) to evaluate potential autonomic nervous system alterations associated with the stress protocol. In addition, these parameters were measured for three days preceding the stress protocol so that pre- and post-challenge responses could be evaluated. This design provided additional insight into the impact of ExHS (compared to heat stress alone) on physiological (and behavioral) responses in these animals.

    For each of the 11 protocol days (baseline, chronic stress, recovery), clean 1-h BP segments were obtained during the active nighttime period (to avoid confounding effects of protocols performed during daytime hours). From these 1-h BP recordings the following parameters were determined:

    - Mean values for systolic, diastolic, and mean BP, HR, and T_c.
    - Low frequency (LF) systolic blood pressure variability as an index of sympathetic modulation of vascular tone.
    - LF and high frequency (HF) heart rate variability as indices for sympathetic and parasympathetic modulation of cardiac function, respectively.
    - Time domain HR variability parameters SDNN and RMSSD as markers for overall (sympathetic and parasympathetic) and parasympathetic mediated HR variability, respectively (note: low values indicate high cardiovascular risk).

- Statistics: Statistical analysis of data was performed by two-way Analysis of Variance (ANOVA) for one repeated (time) and one independent (groups) measure.

- **Results**

For the **clonidine** experiments, studies were completed in 10 HS, 9 ExHS and 10 sham control animals. For the **lisinopril** experiments, studies were completed in 10 HS, 9 ExHS and 9 sham-control animals. Presented below are the physiological data collected and analyzed to date for these studies.

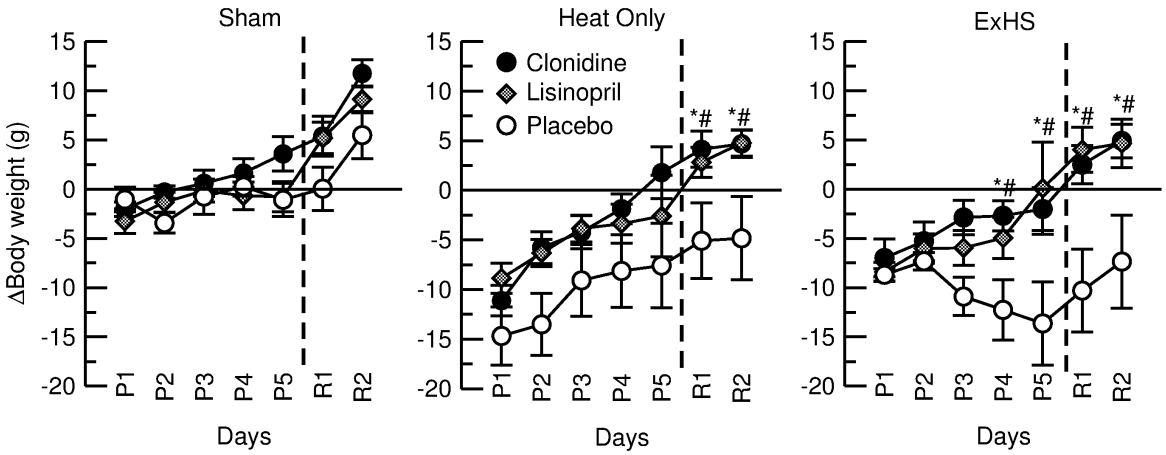

Fig. 1: Time course of changes in body weight over the five experimental protocol days (P1-P5) and the recovery period (R1-R2). After the first protocol day (P1), animals in the heat-only and ExHS groups lost 5-20 g of body weight. This weight loss was not recovered at the end of the recovery period in placebo treated rats. In contrast, body weight in rats treated with clonidine or lisinopril fully recovered.

*:placebo vs. clonidine; #:placebo vs lisinopril.

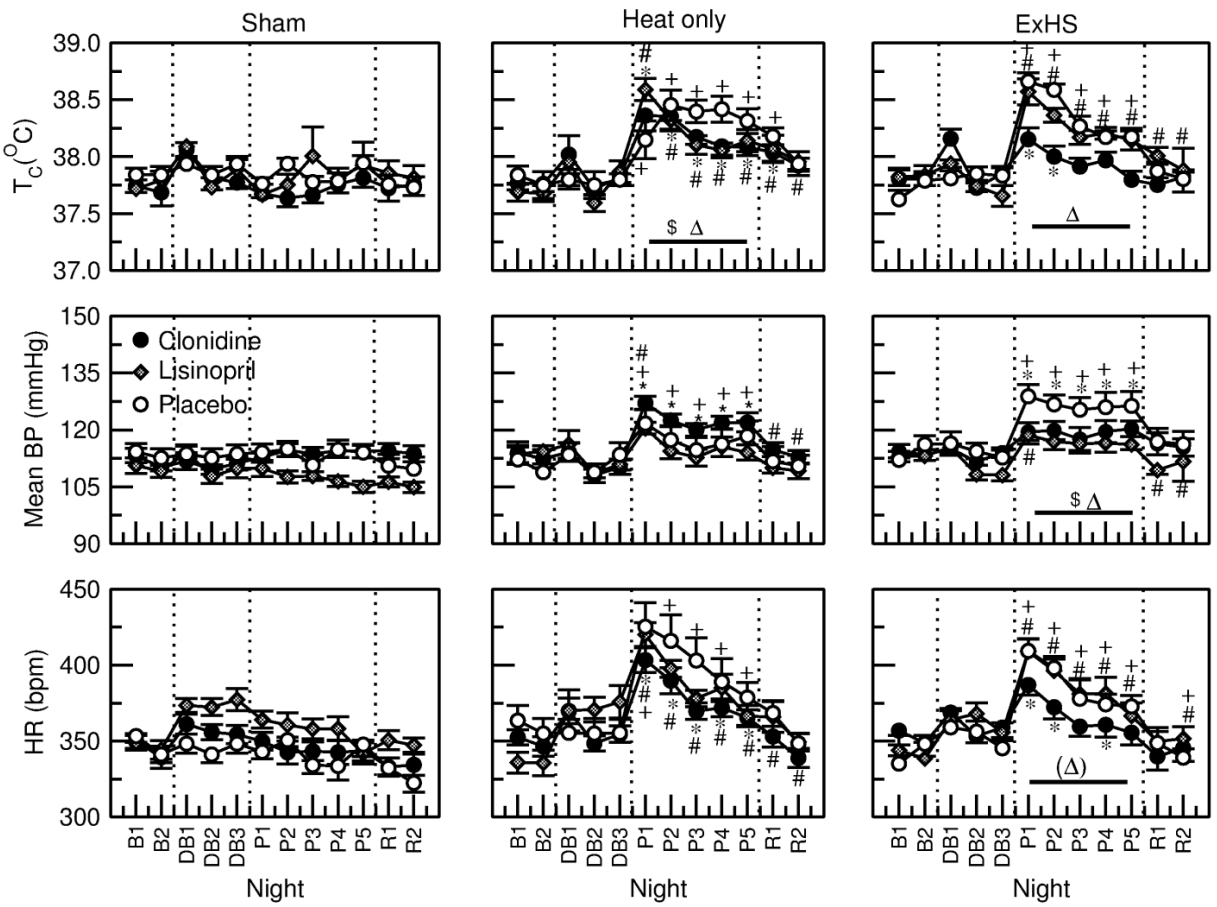

Fig. 2: Time course of body core temperature (T_c), mean blood pressure (Mean BP), and heart rate (HR) in placebo-, clonidine-, and lisinopril-treated rats. Values are obtained during the nighttime to avoid confounding effects of the study protocols conducted during the daytime period. B1-B2: baseline before drug treatment; DB1-DB3: baseline with drug treatment; P1-P5: protocol days 1 to 5; R1-R2: recovery days 1 to 2.

In placebo-treated rats, both protocols (heat only and ExHS) were associated with increases in T_c, HR, and mean BP compared to the preceding baseline (DB3). In the heat-only protocol, both drugs improved thermoregulation as indicated by a gradual decline in nocturnal T_c over the five experimental protocol days that was not observed in placebo-treated rats. In the ExHS protocol, only clonidine improved thermoregulation as indicated by the smaller increase in nocturnal T_c during the five protocol days compared to placebo- or lisinopril-treated rats. The HR responses mirrored the T_c responses, probably because these two parameters are linked through metabolic rate. Mean BP in placebo-treated rats increased slightly in the heat-only protocol and this small increase in mean BP was not affected by the drugs. In contrast, the ExHS protocol caused a pronounced increase in mean BP in placebo-treated rats that was blunted by both the antihypertensive drugs.

*:DB1 vs. clonidine; #: DB1 vs. lisinopril; +: DB1 vs. placebo; Δ: placebo vs. clonidine; $: placebo vs. lisinopril; Δ: $p<0.05$; (Δ): $p<0.1$

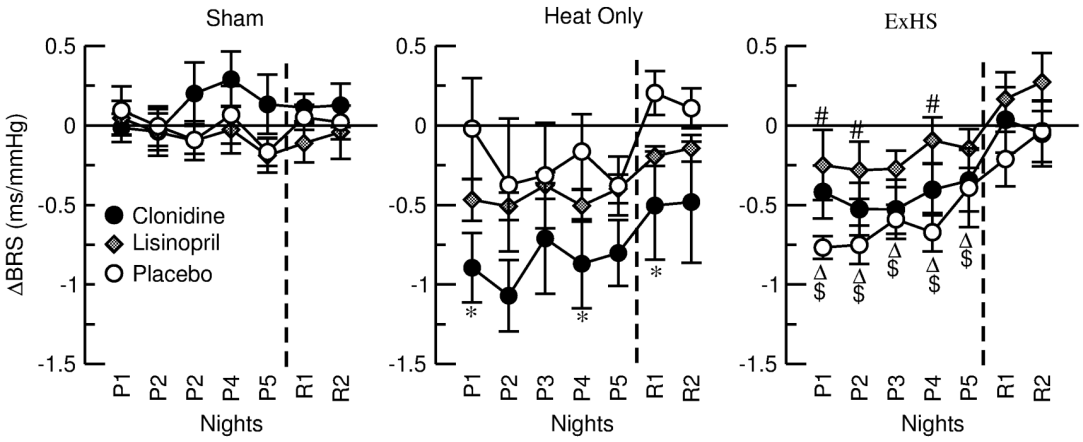

Fig. 3: Time course of changes in baroreceptor-heart rate reflex sensitivity (BRS) during the five experimental protocol days (P1-P5) and during the recovery (R1-R2). BRS was calculated from nighttime recordings to avoid confounding effects of the study protocols conducted during the daytime. Values are the changes from the night before the first protocol day. ExHS, but not heat-only, reduced BRS in placebo-treated rats, suggesting impaired cardiovascular regulation. **Lisinopril improved baroreflex sensitivity in rats exposed to ExHS, suggesting improved cardiovascular regulation**. Angiotensin II is known to suppress baroreflex function. Thus, the effect of lisinopril may be related to inhibition of the angiotensin-mediated suppression of BRS. The heat-only and ExHS protocols both reduced BRS in clonidine-treated rats. This effect of clonidine may be related to the centrally mediated sympatholytic effect of the drug.

*:DB1 vs. clonidine; #: DB1 vs. lisinopril; +: DB1 vs. placebo; Δ: placebo vs. clonidine; $: placebo vs. lisinopril; Δ: $p<0.05$; (Δ): $p<0.1$.

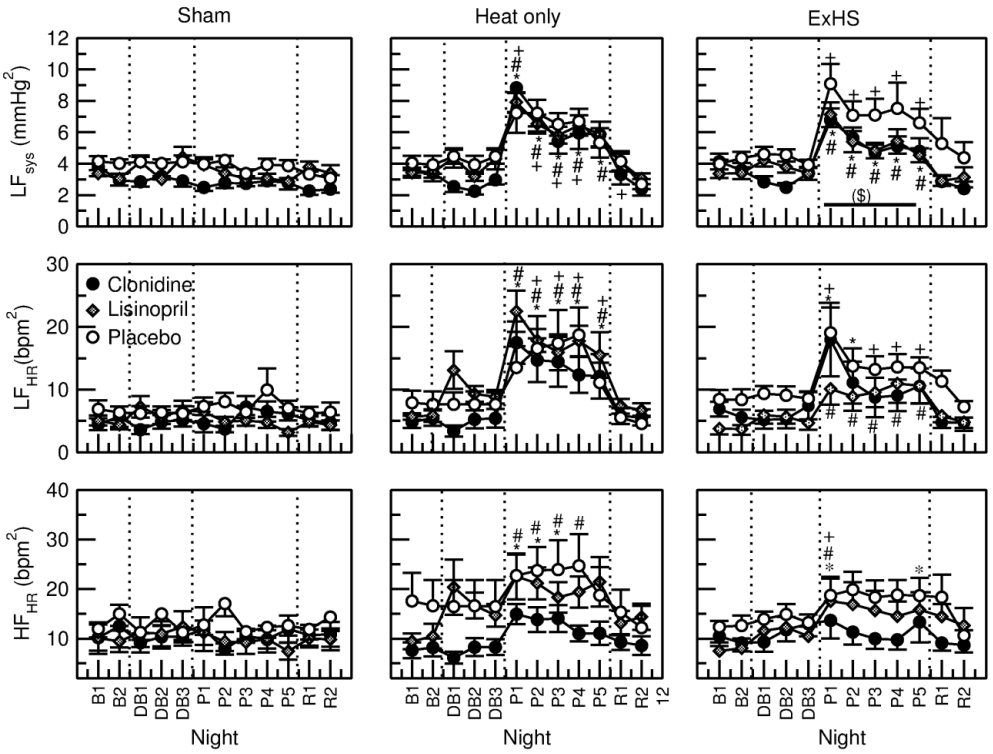

Fig. 4: Low frequency (LF) systolic blood pressure variability (LF_{SYS}), LF heart rate variability (LF_{HR}), and high frequency heart rate variability (HF_{HR}) in placebo-, clonidine-, and lisinopril-treated rats. Values are obtained during the nighttime to avoid confounding effects of the study protocols conducted during the daytime. B1-B2: baseline before drug treatment; DB1-DB3: baseline with drug treatment; P1-P5: protocol days 1 to 5; R1-R2: recovery days 1 to 2. In placebo-treated rats, there was a pronounced increase in LF_{SYS} and LF_{HR} during both experimental protocols (heat only and ExHS), indicating enhanced sympathetic modulation of vascular and cardiac function, respectively. Neither clonidine nor lisinopril changed these responses to the heat-only protocol. However, both drugs reduced the impact of **the ExHS protocol on sympathetic modulation of vascular tone (LF_{SYS}).** High frequency heart rate variability (HF_{HR}) usually is considered a marker for parasympathetic modulation of cardiac function. However, in the experimental protocols of this study, HF_{HR} may be confounded by thermoregulatory changes in respiratory function (panting), and therefore, is difficult to interpret.

*:DB1 vs. clonidine; #: DB1 vs. lisinopril; +: DB1 vs. placebo; $: placebo vs. lisinopril & clonidine; ($): $p<0.1$

- **Summary of Key Results from Task 2**
    - Both clonidine and lisinopril treatments blunted the loss in body weight during chronic ExHS and improved recovery the body weight faster following ExHS compared to placebo-treated rats exposed to ExHS.
    - Lisinopril treatment blunted the decline in baroreflex sensitivity during ExHS compared to placebo-treated rats exposed to ExHS.
    - An analysis of the relationship between HR and core temperature responses during the chronic testing protocol demonstrated that clonidine-treated rats exposed to ExHS protocol had lower heart rate values at a given core temperature than placebo-treated rats. The clonidine-treated animals also presented evidence of heat acclimation from day three on in the protocol.
    - Exploratory behavior (open field tests) declined during the 5 protocol days in ExHS and HS (compared to controls), likely due to fatigue/stress conditions associated with the protocol.

4. KEY RESEARCH ACCOMPLISHMENTS

- Experiments associated with Task 1 were completed, and the results suggest that chronic exposure to ExHS leads to a sustained increase in vascular sympathetic activity despite aerobic training effects and heat acclimation.

- Task 2 experiments are were also completed as proposed. Results from these studies have been presented at national and regional scientific meetings, and manuscripts are being prepared for submission to peer-reviewed journals.

5. CONCLUSIONS

The funded studies have yielded some exciting results that fit well with the hypotheses presented in the project proposal.

Specifically, results from Task 1 studies indicated that chronic exposure to ExHS can lead to a sustained increase in cardiac and vascular sympathetic activity in rats, despite indications that these animals are becoming aerobically trained and heat acclimated as a result of repeated exposure to the heat and exercise test protocol. This response may contribute to autonomic nervous system and cardiovascular dysfunction with a chronic stressor such as ExHS, as suggested by reductions in heart rate variability indices that were observed in exertionally heat stressed group.

Results from Task 2 studies indicate that clonidine and lisinopril may be agents that are beneficial in blunting the weight loss that can accompany a chronic exertional heating challenge. In addition, the reduction in baroreflex function (reflecting autonomic nervous system dysfunction) that accompanies ExHS may be mitigated via treatment with lisinopril. Finally,

clonidine treatment appears to provide some thermoregulatory benefits and enhances heat acclimation in response to chronic ExHS.

"So What" Section: As an overall conclusion to the project, we have demonstrated that a chronic stress protocol (exertional heat stress for five days), which was modeled to have similarities to conditions faced by Warfighters, produces autonomic nervous system and cardiovascular alterations that could have deleterious consequences. Interestingly, these responses are manifested despite indications that the chronic stress protocol does produce some exercise training and heat acclimation effects. We have also observed that widely prescribed blood pressure lowering medications may be beneficial in improving autonomic, cardiovascular, thermoregulatory and behavioral adjustments associated with exertional heat stress. These types of pharmacological treatments should have relevance and applicability to situations encountered by Warfighters.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS

A. Publications/Abstracts

i. Manuscripts

Two manuscripts are in final draft stages and it is anticipated that these manuscripts will be submitted to peer-reviewed journal in the near future.

a. Stauss HM, Choudhary N, and Kregel KC Cardiovascular, autonomic, and thermoregulatory effects of repeated exertional heat stress in rats. In preparation.

b. Stauss HM, Choudhary N, and Kregel KC. Differential effects of clonidine and lisinopril on thermoregulatory and cardiovascular function during chronic exposure to exertional heat stress in rats. In preparation.

ii. Abstracts

a, Stauss HM, Choudhary N, Nash A, Liaboe FO, and Kregel KC. Cardiovascular, autonomic, and thermoregulatory effects of repeated exertional heat stress in rats. (Presented at the 2012 Experimental Biology Meeting, San Diego, April 2012.)

b. Choudhary N, Moss AM, Kregel KC and Stauss HM. Clonidine treatment improves thermoregulatory and autonomic responses to exertional heat stress and may contribute to reduced fatigue and faster recovery. (Presented at the 2012 Iowa Physiological Society Meeting, October 2012.)

c. Stauss HM, Choudhary N, Moss AM, and Kregel KC. Improved thermoregulatory and autonomic responses contribute to reduced fatigue and faster recovery from exertional heat stress by sympatholytic treatment with clonidine. (Presented at the 2013 Experimental Biology Meeting, Boston, April 2013.)

d. Haak JL, Bloomer SA, Stauss HM, Liaboe FO, and Kregel KC. Differential HSP expression in liver and quadriceps in response to chronic exertional heat stress. (Presented at the 2013 Experimental Biology Meeting, Boston, April 2013.)

e. Stauss HM, Choudhary N, Moss AM, and Kregel KC. Differential effects of clonidine and lisinopril on thermoregulatory and cardiovascular function during chronic exposure to exertional heat stress in rats. (Presented at the 2014 Experimental Biology Meeting, San Diego, April 2014.)

B. Presentations made during the last year

i. Military Operational Medicine Research Program (MOMRP) Meeting, Fort Detrick, MD, April 2014: Mechanisms of Autonomic Dysfunction Associated with Extreme Exertional Heat Stroke and Potential Efficacy of Novel Pharmacological Treatments.

ii. Experimental Biology Meeting, San Diego, CA, April 2014: Differential Effects of Clonidine and Lisinopril on Thermoregulatory and Cardiovascular Function During Chronic Exposure to Exertional Heat Stress in Rats.

7. INVENTIONS, PATENTS AND LICENSES

N/A

8. REPORTABLE OUTCOMES

This study clearly demonstrates that clonidine reduces thermoregulatory, hemodynamic, autonomic, and cognitive/behavioral adverse effects associated with exposure to exertional heat stress. There is the hope that our data will translate to clinical and/or military-operational settings, which has the potential to make meaningful contributions in the following areas:

a. Improved performance of Warfighters exposed to chronic exertional heat stress.

b. Reduced casualties and/or lost-man hours related to heat stroke or heat exhaustion.

9. OTHER ACHIEVEMENTS

N/A

10. REFERENCES

See #6 for list of manuscripts in draft form and abstracts that have been presented at national scientific meetings.

11. APPENDICES

APPENDIX I: ABSTRACTS

1. Abstract Presented at the 2012 Experimental Biology Meeting (April 2012)

Cardiovascular, autonomic, and thermoregulatory effects of repeated exertional heat stress in rats

Harald M. Stauss, Navita Choudhary, Abigail Nash, Frederick Liaboe, and Kevin C. Kregel
Department of Health & Human Physiology
The University of Iowa, Iowa City IA

A single bout of exercise in the heat (ExHS) produces specific alterations in autonomic function. This study aimed to investigate the effects of multiple bouts of ExHS (10 ExHS bouts over 5 days; 2x/day). Rats (n=8) were run on a motorized running wheel (7-11 m/min) at 39˚C until core temperature (T_c) reached 41.8˚C. Blood pressure (BP), heart rate (HR) and T_c were recorded telemetrically for 3 days before, during, and 3 days after the ExHS protocol. Both low frequency (LF) HR variability (HR_{LF}) and LF systolic BP (SBP) variability (SBP_{LF}) increased from baseline values in the night following the first protocol day and remained elevated during the nights following the remaining 4 days of ExHS. Compared to baseline (and controls), HR and T_c were temporarily elevated in the night following the first protocol day (368±11 vs. 317±4 bpm and 38.8±0.04 vs. 37.7±0.08^{o}C) but returned to baseline in the night following the fifth protocol day, suggesting a training and heat acclimation effect. HR-related measures suggested no changes in cardiac parasympathetic modulation occurred with ExHS. The elevated nocturnal HR_{LF} and SBP_{LF} throughout the ExHS protocol, together with the blunted HR and T_c responses at the end of the ExHS protocol, suggest that chronic exposure to ExHS can lead to a sustained increase in cardiac and vascular sympathetic activity despite training and heat acclimation.

Supported by DoD/USAMRMC #W81XWH-10-2-0115.

2. Abstract Presented at the 2012 Iowa Physiological Society Meeting (October 2012)

Clonidine Treatment Improves Thermoregulatory and Autonomic Responses to Exertional Heat Stress and may Contribute to Reduced Fatigue and Faster Recovery

Navita Choudhary, Anne M. Moss, Kevin C. Kregel, and Harald M. Stauss
Department of Health and Human Physiology
The University of Iowa, Iowa City, IA, USA

In previous studies we demonstrated that repeated exposure to heat stress (HS, 45oC ambient temperature) or exercise (7-11 m/min on running wheel) in the heat (ExHS, 39oC ambient temperature) elicits characteristic hemodynamic, thermoregulatory, and autonomic responses in rats. In the current study we investigated the effect of the sympatholytic drug clonidine on these responses to exertional (ExHS) or non-exertional (HS) heat stress. Blood pressure (BP), heart rate (HR) and core temperature (Tc) were continuously recorded telemetrically for 5 days before

(baseline), during, and 3 days after (recovery) a 5-day ExHS, HS, or sham (thermoneutral conditions, no exercise) protocol. Exploratory behavior that is affected by factors such as anxiety and fatigue was assessed using an open field test. Clonidine (33 μg/kg/day) or placebo was continuously administered via osmotic minipumps starting 3 days before the first ExHS, HS, or sham protocol day. On the protocol days, rats were exposed to ExHS, HS, or sham conditions twice a day. Experimental conditions were terminated once Tc reached 41.8oC (ExHS and HS) or after 90 min (sham, average ExHS duration). In the nights following each of the five protocol days, Tc and HR were significantly elevated in placebo-treated rats exposed to ExHS and HS compared to sham. Clonidine reduced these increases in Tc and HR, suggesting improved thermoregulation. Compared to sham, nocturnal BP values in placebo-treated rats were more elevated in the ExHS than in the HS group. Clonidine reduced nocturnal BP in ExHS but not in HS, suggesting that the nocturnal hypertension in placebo-treated rats exposed to ExHS is related to increased sympathetic tone. In line with this observation, in placebo-treated rats, low frequency (LF) systolic BP variability, reflecting sympathetic modulation of vascular tone, was more elevated in the ExHS than in the HS group. Furthermore, clonidine reduced the elevated LF systolic BP variability in the ExHS but not in the HS group. Compared to baseline, exploratory behavior declined during the 5 protocol days in ExHS and HS, which was likely related to fatigue. In ExHS, this decline in exploratory behavior was blunted by clonidine treatment. Furthermore, recovery of exploratory behavior in ExHS was more pronounced and faster in clonidine- compared to placebo-treated rats. We conclude that clonidine treatment improves thermoregulation and reduces sympathetic responses to chronic exposure to exertional heat stress. These beneficial effects of clonidine may reduce fatigue and contribute to faster recovery following chronic exposure to exertional heat stress as indicated by the improved exploratory behavior in the open field test. Supported by funding from DoD/USAMRMC #W81XWH-10-2-0115.

3. Abstract Presented at the 2013 Experimental Biology Meeting (April 2013)

Improved Thermoregulatory And Autonomic Responses Contribute To Reduced Fatigue And Faster Recovery From Exertional Heat Stress By Sympatholytic Treatment With Clonidine

Harald M. Stauss, Navita Choudhary, Anne M. Moss, and Kevin C. Kregel
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We investigated the effect of clonidine (C, 33 μg/kg/day, osmotic pumps) on thermoregulatory and autonomic responses to chronic exposure to exertional (ExHS) and non-exertional (HS) heat stress in rats. Blood pressure (BP), heart rate (HR) and core temperature (T_c) were recorded telemetrically before, during, and after a 5-day (twice a day) ExHS, HS, or sham protocol. During the five protocol days, nocturnal T_c and HR were elevated in placebo-treated rats (P) exposed to ExHS and HS. C reduced these increases in T_c and HR, suggesting improved thermoregulation. Nocturnal BP in P was more elevated in ExHS than in HS. C reduced nocturnal BP in ExHS, suggesting increased sympathetic tone in P exposed to ExHS. Similarly, in P, low frequency systolic BP variability (LF_{SYS}), reflecting sympathetic modulation of vascular tone, was more elevated in ExHS than in HS and C reduced this elevated LF_{SYS} in

ExHS. Likely due to fatigue, exploratory behavior (open field test) declined during the 5 protocol days in ExHS and HS. In ExHS, this effect was blunted by C. Furthermore, recovery of exploratory behavior in ExHS was more pronounced and faster in C-treated rats compared to P. C improves thermoregulation and reduces sympathetic responses to chronic exposure to ExHS. These effects of C may reduce fatigue and contribute to faster recovery following chronic exposure to ExHS. Supported by funding from DoD/USAMRMC #W81XWH-10-2-0115.

4. Abstract Presented at the 2013 Experimental Biology Meeting (April 2013)

Differential HSP expression between liver and quadriceps in response to chronic exertional heat stress

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Heat shock proteins (HSP) are upregulated as a key component of the cellular stress response to physiological challenges such as heat and exercise. In this experiment, we investigated the HSP response to the combined stressor of exercise plus hyperthermia in a chronic exertional heat stress (ExHS) model. In the ExHS experiments, male Sprague-Dawley rats were run at 7–11 m/min in an environmental chamber (39°C) until core temperature (Tc) reached 41.8°C. Animals were exposed to 2 bouts of exertional heat stress per day for 5 consecutive days. In the passive heat stress experiment (HS), animals were placed in the chamber (45°C) until Tc reached 41.8°C. Nonheated animals served as controls. Three days after the final bout of ExHS or HS, rats were euthanized, and livers and quadriceps were harvested. HSPs were evaluated on whole-tissue homogenates by immunoblotting. In the quadriceps and liver, HSP70 was barely detectable in the nonheated condition; however it was elevated in both tissues in response to HS and ExHS. Interestingly, liver in the the ExHS group displayed a 77% greater expression of HSP70 and a 35% greater expression of HSP60 compared to the HS group. However, in the quadriceps expression of HSP60 and HSP70 did not differ between HS and ExHS. Compared to the quadriceps, this augmented response of the liver to exertional heat stress suggests greater perturbation to homeostasis in the liver after ExHS. Supported by DoD/USAMRMC #W81XWH-10-2-0115.

5. Abstract Presented at the 2014 Experimental Biology Meeting (April 2014)

Differential Effects of Clonidine and Lisinopril on Thermoregulatory and Cardiovascular Function During Chronic Exposure to Exertional Heat Stress in Rats

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The effects of clonidine (C, sympatholytic drug) and lisinopril (L, ACE inhibitor) on thermoregulatory, cardiovascular, and autonomic responses to chronic (5 days, 2 times/d) exertional (ExHS) or non-exertional (HS) heat stress were assessed in rats. ExHS and HS elicited increases in nocturnal core temperature (T_C) and heart rate (HR) in placebo (P) treated rats. This effect was blunted by C in the ExHS protocol and by both drugs in the HS-only protocol.

Nocturnal blood pressure (BP) increased more during the ExHS than the HS protocol. This hypertensive response to ExHS was blunted by both drugs. In P-treated rats, baroreflex sensitivity decreased only during the ExHS protocol. L improved this impaired baroreflex function during exposure to ExHS more than C. In P-treated rats, sympathetic modulation of cardiac and vascular function (low frequency HR and BP variability) was elevated in both protocols. Both drugs reduced this elevated sympathetic modulation of cardiovascular function. In conclusion, C and L elicited beneficial thermoregulatory (T_C), cardiovascular (BP, baroreflex function), and autonomic (cardiac and vascular sympathetic modulation) effects during exposure to HS or ExHS. However, during exposure to ExHS, C appears to be more effective in improving thermoregulation than L, while L may be more effective in improving cardiovascular (baroreflex) function than C. Supported by DoD/USAMRMC #W81XWH-10-2-0115.